LOW MOLECULAR WEIGHT HEPARIN

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UFH was not. In this study, the weight of thrombus formed was measured 6 hours after total ligation of the vena cava, without any induction of hypercoagulability. UFH over a wide dose range (0.2–1.66 mg/kg) appeared to have no effect in limiting the extension of an existing thrombus whereas the LMW heparin at the highest dose studied (1.66 mg/kg) did limit thrombus extension. However, this is clearly a different model from those discussed earlier, and the pathogenetic mechanisms also appear to be different. It seems reasonable to conclude, as do the authors, that other factors are involved in their model, such as enhanced thrombolytic activity by LMW heparin. A model in which UFH (at what would be considered high dosage in other models) has no measurable antithrombotic effect raises questions as to the validity of this approach for the study of the prevention of venous thrombosis.

In another animal model (baboons), measuring thrombus formation in an exteriorised femoral arteriovenous shunt, Cadroy et al (38) compared the relative antithrombotic and antihaemostatic effects of a very low MW heparin (CY 222) and UFH. The device they used consisted of a collagen-coated cannular segment positioned proximal to two regions of expanded diameter exhibiting disturbed flow and stasis, while thrombus formation was measured using indium-labelled platelets. Both heparin preparations abolished thrombus formation in low-shear fibrin-rich regions at plasma levels less than 0.5 anti-Xa units/ml, but platelet deposition onto the collagen surface was not reduced by either drug at that dosage. To reduce platelet deposition, anti-Xa levels of 1–5 anti-Xa units/ml were needed. Cadroy et al commented that although equivalent antithrombotic effects were achieved for platelet-dependent thrombus formation at comparable anti-Xa levels, UFH prolonged both the coagulation time and the bleeding time substantially more than did CY 222. Thus, in terms of platelet-dependent thrombotic events, LMW heparin (CY 222) showed a much more favourable ratio of antithrombotic to antihaemostatic effects than did UFH. In terms of arterial thrombosis, where the dose required was up to 10 times that needed to block the formation of static, fibrin-dependent thrombi, it is clearly of considerable advantage to use a drug with diminished effect on the haemostatic system.

LMW HEPARIN AND EXPERIMENTAL THROMBOSIS

The first reports on the effect of LMW heparin in animals were published (14, 23) 5 years after the initial report of its use in human volunteers (39). This surprising reversal of the usual situation, with studies on animals before human experiments, presumably reflected the fact that heparin was a well-known drug and that therefore a heparin derivative was not thought to need animal toxicology studies. Nevertheless, extensive

toxicological studies have been carried out on all the current LMW heparins on the market, reflecting the more rigorous licensing requirements of today, and perhaps also the fact that current LMW heparins are usually not straightforward fractions of commercial UFH. The initial human volunteers and patient studies were carried out by a small group of research workers in London in the period 1976 to 1982 (14, 39–42).

The first study showing that LMW heparin was capable of preventing experimental venous thrombogenesis was that of Thomas et al (14). The material they used was prepared commercially by Glaxo from porcine mucosal heparin by alcohol extraction, and also had a relatively high mean MW of 8000–9000. The fraction preserved the anti-Xa activity, but had only half the specific activity of UFH by APTT assay. In the Wessler stasis model, with human serum as a thrombogenic stimulus, this heparin fraction completely inhibited thrombus formation at a dose of 35 μg/kg. Over the dose range studied, the fraction was fully as effective as UFH on a weight basis, probably due to the fact that this LMW heparin retained significant anti-IIa activity, with an anti-Xa/anti-IIa ratio of 2. It is a matter of some regret (at least to British eyes) that this early development of a LMW heparin was not pursued by Glaxo, despite a promising start. It was left to our French colleagues to exploit the therapeutic possibilities of LMW heparin, and to market successfully the first LMW heparin.

In 1981, Carter et al (23) reported the comparative efficacy of an unfractionated porcine mucosal heparin (specific activity 168 USP units/mg) and a depolymerised LMW heparin of mean MW 9200 (specific activity 91 USP units/mg). Antithrombotic effectiveness was determined by measuring their ability in the rabbit to inhibit labelled fibrinogen accretion on a standardised preformed venous thrombus. The thrombus was formed by injecting thrombin into an isolated venous segment, removing the ligature and restoring blood flow around the thrombus. A constant infusion of heparin or LMW heparin was then maintained for 7 hours, after which the jugular thrombus was removed and the amount of fibrinogen accreted onto the preformed thrombi calculated by comparing the specific activity of the rabbit's fibrinogen with the radioactivity of the thrombi. Both heparins produced a significant reduction in the amount of fibrinogen accreted onto experimental thrombi when compared to a saline control. In the control animals, the geometric mean accretion was 156 μg whereas in the case of UFH it was 17 μg and for LMW heparin it was 6 μg. In the treated animals, both sets received approximately the same amount of heparin in terms of USP unitage (425 units for UFH and 403 units for LMW heparin). Two important points should be noted about this study. Firstly, the end-point measured was not inhibition of thrombus formation but the reduction of fibrin accretion on a preformed thrombus. Secondly, in terms of dry weight, more LMW heparin was given than UFH (425/168 = 2.53 mg in the case of UFH, and 403/91 = 4.43 mg in the case of LMW heparin). These

considerations must be taken into account when assessing the authors' claim that the LMW heparin they used had "a significantly greater antithrombotic effect" than UFH. It would be equally valid to say that almost twice as much LMW heparin by weight was required to produce a broadly comparable antithrombotic effect (a reduction of 89% in the case of UFH and 96% in the case of LMW heparin).

In a subsequent study, using the same model, but a much smaller dose of heparin/LMW heparin, and only waiting 15 minutes before examining the thrombi, the authors came to the same conclusion, namely that LMW heparin possessed greater antithrombotic activity than UFH – in this case, a LMW heparin of lower MW (4600) and lower USP potency (41 units/mg) (43). As would be expected, the amount of fibrin accretion was much lower in this second study, falling from 71.2 μg in saline controls to 14.6 μg for UFH and 5.2 μg for LMW heparin. These differences are non-significant, although clearly there is a trend towards both drugs reducing thrombus size. When the dose of UFH was reduced to 10 USP units/kg, there was no difference between controls and UFH (81.6 and 80.5 μg respectively), but the LMW heparin animals had thrombus accretions of 24.6 μg, and this difference was significant at the 5% level. The amount of LMW heparin administered in this study on a dry weight basis was again considerably higher than the amount of UFH. The specific activity of UFH used was 145 units/mg, whereas that of the LMW heparin was 41 units/mg. It can be calculated that 145/41 = 3.5 times as much LMW heparin was given by weight when administering 20 USP units/kg of either drug. The claim made by the authors (and frequently quoted in the literature) that LMW heparin shows greater antithrombotic activity than UFH must be heavily qualified by noting that this is only true in the context of administering LMW heparin on the basis of USP units (or other pharmacopoeial or "global" units), and not by dry weight. If the LMW heparin had been administered on a weight basis the results would have been quite different, and UFH would have been more effective than the LMW heparin studied. The other claim in this paper (43), that LMW heparin caused less blood loss than UFH, is discussed elsewhere (p. 146).

Cade et al (24) compared the antithrombotic and haemorrhagic effects of two LMW heparin fragments and a heparinoid with porcine mucosal heparin, and related their in vivo findings to the results of ex vivo tests of blood coagulation and in vitro tests of platelet function. They assessed the antithrombotic effects by measuring inhibition of a tissue thromboplastin-induced jugular vein thrombus, and also the inhibition of fibrin and platelet accumulation in an arteriovenous shunt. Their results confirmed the earlier finding that, for an equivalent antithrombotic effect, the LMW heparins studied produced less haemorrhage than UFH (23). In addition, the antithrombotic effects of all the four GAGs studied occurred at similar levels of anti-Xa activity (0.1–0.2 units/ml). However, they found no relationship

between blood loss and the effects of the GAGs studied on the anti-Xa values, on the APTT, or on the thrombin clotting time measured ex vivo. UFH was found to have a greater inhibitory effect on collagen-induced platelet aggregation than the LMW GAGs, suggesting that the increased bleeding observed with UFH may in part be related to its inhibitory effect on platelet function.

EFFECT OF METHOD OF PREPARATION

Ostergaard and colleagues (44) carried out an important study in which they examined three LMW heparins prepared by enzymatic depolymerisation, chemical degradation and fractionation, respectively. All three LMW heparins had comparable MW distributions, with a peak MW of around 5000, and very similar in vitro activities (anti-Xa/APTT ratios of 1.8–2.1). In rabbits, no differences were found in antithrombotic effectiveness between any of the three LMW heparins compared to each other or to UFH, at a dose of 30 anti-Xa units/kg. Similarly, neither LMW heparin nor UFH (60 or 90 anti-Xa units/kg) prolonged haemostatic plug formation time in the mesenteric microcirculation. The authors concluded that, except for decreases in the chain length, various manufacturing processes do not give rise to essential structural changes in the heparin molecules, and commercial LMW heparins of similar MW distributions have virtually the same characteristics in vitro and in vivo.

In contrast to these results, Doutremepuich et al (45) studied seven LMW heparins, and compared their in vitro and in vivo (subcutaneous) antithrombotic properties. The animal model used was the rat, tying off the inferior vena cava (IVC). Two hours later the rats received a subcutaneous injection of heparin, LMW heparin or placebo (saline). The doses of UFH or LMW heparin were given on an equivalent weight basis (1 mg/kg). Six hours after ligation the clot was removed from the IVC, dried and weighed. The authors showed that the weight of the clot varied considerably depending on the LMW heparin employed, with UFH having no significant antithrombotic effect, and CY 222 having the most potent effect. The authors concluded that the mode of industrial preparation and the molecular structure play a major role in the antithrombotic effectiveness of LMW heparins. However, there was considerable variation between different batches of the same LMW heparin, and the question at issue with this study is whether the results truly represent variations in antithrombotic effectiveness of various LMW heparins, or whether the poor reproducibility of the model is largely responsible for the apparent differences observed.

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Clinical Studies in Prophylaxis of Thrombosis

DEEP VEIN THROMBOSIS (DVT)

In 1988, Collins et al (1) published an overview of results of randomised trials in general, orthopaedic, and urological surgery, and concluded that there was a reduction in fatal pulmonary embolism and venous thrombosis by perioperative administration of subcutaneous heparin (UFH). In a review of more than 70 randomised trials in 16 000 patients, they concluded that the use of subcutaneous heparin can prevent about half of all pulmonary emboli and about two thirds of all DVTs. The reduction in deaths attributed to pulmonary embolism was striking, with 19 deaths in patients receiving heparin, as compared to 55 deaths in control patients ($P<0.001$). This reduction in mortality was not offset by any increase in deaths due to other causes, and therefore total mortality was also reduced significantly. They found no evidence that the more convenient 12-hour regimen of subcutaneously administered heparin produced any less of an effect on pulmonary embolism than an 8-hourly regimen favoured originally and concluded that a fixed dose of subcutaneous heparin administered every 12 hours will prevent about half the pulmonary emboli occurring after many types of surgery, without producing any substantial increase in serious bleeding. However, in their meta-analysis, the authors noted clear and consistent evidence for an increased risk of bleeding after administration of subcutaneous heparin (overall, 419 episodes in the heparin group versus 244 in the control group) in some 13 500 patients for whom bleeding data were available. The number of fatal haemorrhages was 8 in the heparin group and 6 in the control group.

This impressive analysis of the evidence for the protective effect of low-dose subcutaneous heparin, when used following general and orthopaedic surgery, provides a useful background against which LMW heparin can be compared. If a twice-daily injection of 5000 IU of UFH is considered "standard therapy" for the prophylaxis of postoperative DVT, how can

these results be improved upon? There are only three main ways in which this could happen, namely LMW heparin could be safer (less bleeding), more effective (fewer DVTs and pulmonary embolisms) or more convenient (less than two injections a day). The extent to which LMW heparins have achieved these goals is still a matter of debate. In a recent meta-analysis carried out by a group in the Netherlands (2), 62 studies with a total of over 20 000 patients were examined. All studies were randomised, with a control treatment of placebo ($n = 12$), standard heparin ($n = 44$) or dextran/warfarin ($n = 6$). The patients receiving prophylaxis were undergoing general surgery ($n = 35$), orthopaedic surgery ($n = 24$) or stroke ($n = 3$). Prophylaxis with LMW heparin was associated with a 15% increase in the number of major bleedings (95% confidence limits, 0.8–1.5). The authors pointed out that the better studies yielded a higher bleeding risk and a smaller protective effect, and they concluded that overall LMW heparin did not represent a major improvement in the benefit–risk ratio for prophylaxis of thrombosis in patients undergoing general surgical procedures.

However, the study also found that in orthopaedic patients the absolute benefit is much larger, and LMW heparin can therefore be considered a superior drug to UFH for patients undergoing total hip replacement (see later). This important study represents the most convincing evidence to date that the incidence of haemorrhagic side-effects with LMW heparin is at least as great as with UFH, and should dispel any lingering notions that LMW heparin is in some way a safer drug. What remains incontestable is that a single daily injection of a drug is preferable to two injections, and in this respect LMW heparins are clearly superior to UFH in a prophylactic regimen.

The efficacy and safety of LMW heparin in clinical trials over the past decade will now be considered, with particular emphasis on data that have been obtained from large-scale, prospective, randomised, double-blind trials. The first prospective study of the efficacy of LMW heparin in patients was published by Kakkar et al (3). They injected a heparin fraction prepared by gel permeation (mean MW 6000) into 150 consecutive patients undergoing major abdominal surgery. Fifty of these patients received 1250 units every 12 hours, while the other 100 patients received a single injection of 1850 units daily. The units of activity were measured by an APTT assay, and the LMW heparin had an anti-Xa/APTT ratio of 4. None of the patients died from pulmonary embolism, and 3 in each group developed isotopic evidence of DVT. Although there was no control group, subcutaneous LMW heparin administration was not apparently associated with any increase in preoperative or postoperative bleeding, although prophylaxis was discontinued owing to bleeding complications in 2 of the 50 patients who received injections every 12 hours. The authors also studied the effects of equal amounts of LMW heparin and UFH on the coagulation mechanism during surgery in a further 30 patients. Clotting assays and platelet function

tests indicated that both preparations of heparin produced comparable effects, although intra-group comparisons revealed significant differences in anti-Xa activity, lipoprotein lipase release and plasma prekallikrein and thromboxane B_2 concentrations (3). An important observation in relation to the clotting tests was their finding that the anti-Xa activity during surgery was 0.175 units/ml in the group receiving LMW heparin as opposed to 0.09 units/ml in the group receiving UFH ($P<0.05$), confirming the original observation (4) that LMW heparin gave higher anti-Xa levels than did UFH, some 2 hours after subcutaneous administration. Surprisingly, higher anti-Xa activity in the LMW heparin patients was not observed immediately before surgery or 1 day after operation. This paper (3) was the first to show that a single daily dose of LMW heparin administered subcutaneously was sufficient to prevent postoperative DVT in 97 out of 100 patients monitored by labelled fibrinogen scanning for at least 10 days. While there was no control group, and the basis on which the unitage of the dose was calculated could be criticised, the central fact remains that this pioneering study paved the way for all the many studies that have followed, confirming the original observation that a single daily dose of a LMW heparin prevented most postoperative DVT (3).

In a follow-up study, Kakkar and Murray (5) examined the safety and efficacy of the same LMW heparin (Choay CY 216) in the prevention of DVT in a double-blind, randomly allocated trial, comparing LMW heparin with UFH. Almost 400 patients were entered into the trial, half of whom were treated with UFH and half with LMW heparin. Of the patients receiving UFH, 7.5% developed DVT, as compared to 2.5% of the patients receiving LMW heparin ($P<0.05$). They found no significant difference between the two groups in terms of excessive incisional or total blood loss during surgery. In a further 910 patients included in an "open" study, who received a single injection of LMW heparin every day, 3.4% developed DVT. Thus, in three separate groups of patients treated with LMW heparin (CY 216) (the original 100 patients, the control study in 200 patients, and the "open" study of 900 patients), the incidence of postoperative DVT was constant at about 3% (3, 5). The conclusion was drawn that a single daily injection of a LMW heparin was more effective than 5000 units of UFH given twice a day. In the light of subsequent studies, it is worth noting that Kakkar and Murray (5) reported an incidence of haematoma of only 1.5% in 709 patients undergoing general abdominal surgery, although the incidence was higher in patients undergoing gynaecological surgery. They provided no evidence, and did not claim, that LMW heparin was a safer drug than UFH, but made the reasonable point that a single daily injection was likely to be more acceptable to patients, and certainly saved nursing time.

The original "dose-ranging" study of LMW heparin had given encouraging results (3). However, in 1984, Schmitz-Huebner et al (6) reported the results of a randomised controlled clinical trial in which they